In vitro study of the antimicrobial property of Green tea extract against standard (ATCC) bacterial strains and clinical isolates of Methicillin Resistant *Staphylococcus aureus* & Multidrug Resistant *Pseudomonas aeruginosa*

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Abstract

Objective: To evaluate the antimicrobial activity of Aqueous Green Tea extract against Standard ATCC Strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853) & Clinical Isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) & Multidrug Resistant *Pseudomonas aeruginosa* (MDRPA).

Methods: This Cross-sectional Analytical study was conducted in the Department of Microbiology of a Teaching Tertiary care hospital of Central India over a period of two months after obtaining Ethical clearance. Antimicrobial activity of green tea extract was determined by Agar well diffusion method.

Results & Interpretation: The results of this study has shown that the aqueous extract of Green tea exhibits a potent antibacterial activity. Inhibition Zone diameter of green tea extract at lowest concentration (12.5 mg/ml) was 8mm, 10mm & 10mm respectively for ATCC strains of *E.coli, Pseudomonas aeruginosa* & *Staphylococcus aureus*. However at highest concentration (200mg/ml) it was 20mm, 22mm & 26mm respectively.

Amongst six MRSA isolates tested aqueous green tea extract exhibited significant antibacterial activity against five isolates.

Amongst six MDRPA isolates tested even at lowest concentration of 12.5 mg/ml aqueous green tea extract showed appreciable antimicrobial activity against four isolates.

Conclusion: Aqueous extract of Green tea (*Camellia sinensis*) prepared by cold extraction method do have a significant antibacterial activity not only against standard ATCC bacterial strains but also against highly resistant clinical isolates of MRSA & MDRPA.

Keywords: Camellia sinensis, Green Tea extract, Methicillin Resistant Staphylococcus aureus (MRSA), Multidrug Resistant Pseudomonas aeruginosa (MDRPA), Agar well diffusion, Inhibition zone diameter.

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Introduction

Green Tea (*Camellia sinensis*) leaves are known for their Antibacterial activity against many micro-organisms since 90 years[1,2]

This antibacterial property of Green Tea is mainly attributed to the Polyphenolic Components especially **Catechins** including Epicatechin, Epicatechin gallate, Epigallocatechin and Epigallocatechin gallate.[3]

Control of infections caused by Highly Resistant strains of bacteria has become a major problem. Excessive use and misuse of antibiotics has accelerated the emergence and spread of Multi drug resistant strains. A high percentage of Hospital acquired infections are caused by highly resistant bacteria such as Methicillin Resistant *Staphylococcus aureus*(MRSA) or Multi drug Resistant Gram negative bacilli. Such infections fail to respond to standard treatment resulting in prolonged illness, higher health costs and greater risk of death. With the emergence of multi drug resistance, sooner or later these infections will become untreatable as clinicians will be left with no more alternatives.[4]

Therefore, there is increasing need of developing newer antimicrobial agents to face this challenge. There has been an increasingly growing interest to explore newer antimicrobial compounds from medicinal plant extracts, which could be incorporated in the treatment of such infections. It is well known that unfractionated extracts of Green Tea possess modest Antimicrobial Activity. Since green tea is non- toxic & can be consumed over long periods of time without any known side effects, it's possible role as an adjuvant Therapeutic Agent in managing Bacterial Infections deserves consideration.[5]

In this context, there is a need to evaluate the antimicrobial activity of green tea extract. In Indian scenario, very few studies have been conducted to investigate this property of Green Tea. Keeping this in mind we conducted a Cross sectional Analytical Study to evaluate the antimicrobial activity of Green Tea extract against Standard ATCC Strains & Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*

(MRSA) & Multidrug Resistant *Pseudomonas aeruginosa.*

Materials and Methods

This Cross-sectional analytical study was carried out in the Department of Microbiology attached to a Tertiary care Teaching Hospital of Central India during a time period of three months from 1st August 2015 to 31st October 2015 after obtaining clearance from Institutional ethics committee.

In vitro antibacterial activity of aqueous extract of Green tea (*Camellia sinensis*) was studied against three standard bacterial strains

1. *Staphylococcus aureus* ATCC 25923
2. *Escherichia coli* ATCC 25922
3. *Pseudomonas aeruginosa* ATCC 27853 and

Against 6 clinical isolates each of Methicillin Resistant *Staphylococcus aureus* (MRSA) & Multidrug Resistant *Pseudomonas aeruginosa* by Agar Well Diffusion method**.**

Inclusion criteria

For MRSA: Clinical isolates of *Staphylococcus aureus* derived from the routine microbiological processing of samples sent to our microbiology lab for culture-sensitivity testing which exhibited resistance to the antibiotic disc of Cefoxitin (30 µg) with Inhibition Zone diameter < 24mm by Standard Disc diffusion method as per CLSI guidelines[8].

For MDRPA: Clinical isolates of *Pseudomonas aeruginosa* derived from routine microbiological processing of samples sent to our microbiology lab for culture sensitivity testing which exhibited resistance to at least one antibiotic in > 3 classes of anti Pseudomonal antibiotics by Standard Disc Diffusion method as per CLSI guidelines[8].

1. **Preparation of Aqueous extract of Green tea by Cold extraction method**[7]: Dried leaves of *Camellia sinensis* (Goodricke Barnesbeg Darjeeling Green Tea) was procured from local vendor and pulverized to coarse powder.

10 gm of this powder was extracted by soaking for two days (maceration) in 100 ml. of autoclaved distilled water in a 250ml sterile conical flask, with intermittent shaking at room temperature. The extract was then filtered through a sterile muslin cloth for coarse residue and finally filtered through sterile Whatman No.1 filter paper. The filtrates were then concentrated by using Rotary vaccum evaporator (Equitron-Roteva/India) at 40ºC and stored in an air tight container at 4ºC until use.

2. **Antibiotic sensitivity test**[3,8]: Susceptibility testing of the Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was performed using Standard Disc-diffusion method and results interpreted in accordance with CLSI guidelines.
3. **Antibacterial activity test:** For assessing in-vitro antimicrobial activity of aqueous extract of *Camellia sinensis* against MRSA, MDRPA and Standard bacterial strains (*E coli* ATCC 25922, *S. aureus* ATCC 25923 and *P.aeruginosa* ATCC 27853) by Agar well diffusion method[5,7,9]. Five concentrations of extract were prepared by double dilution (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml) in sterile distilled water.

Test organisms were inoculated in peptone water to achieve a turbidity of 0.5 McFarland unit by using Densimat (Bio-Merieux, India) corresponding to bacterial density of 10^8 cfu/ml. Pre-warmed Mueller-Hinton agar plates were seeded with inoculums to achieve lawn culture with sterile swab sticks. Wells of 6mm diameter were aseptically punched in the MHA plate using cork borer. Five wells at the periphery were loaded with 50 µl each of Green Tea extract at different concentrations and the central well for Negative Control with 50 µl of Sterile distilled water.

The plates were incubated at 37º C for 24 hrs. and thereafter observed for appearance of zones of inhibition around the wells. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in mm).

Results

The results of the study showed that the aqueous extract of *Camellia sinensis* exhibited a potent antibacterial activity. The assessment of antimicrobial activity was based on measurement of diameter of zones of inhibition formed around the wells.

The antimicrobial activity of aqueous extract of Commercial green tea leaves against Standard bacterial strains and Clinical isolates of MRSA & MDRPA is depicted in Tables & Fig. 1, 2, 3 respectively.

Amongst standard bacterial strains Aqueous extract of *Camellia sinensis* has highest antimicrobial activity against *S. aureus* ATCC-25923 followed by *P. aeruginosa* ATCC-27853 and then *E.coli* ATCC-25922. But this difference was appreciable at higher concentrations only. (200 mg/ml & 100 mg/ml). However at low concentrations (50 mg/ml, 25 mg/ml & 12.5 mg/ml) the antimicrobial activity of aqueous green tea extract against the three organisms remained almost same.

Amongst the 6 strains of MRSA, the Aqueous extract of *Camellia sinensis* exhibited a fairly significant antimicrobial activity against 5 strains (MRSA-1, 2, 3, 4, 5). Even at lowest concentration of 12.5 mg/ml the aqueous green tea extract showed antimicrobial activity against four strains (MRSA 1, 2, 3, 5).

In case of 4 strains (MRSA 1, 2, 3, 4), on increasing the concentration of aqueous green tea extract from 12.5 mg/ml to 100 mg/ml there was a significant increase in inhibition zone diameter.

In case of MRSA-6 even with increasing concentration of aqueous green tea extract there was not

much appreciable increase in antimicrobial activity and inhibition zone diameter remained almost same.

In all 6 strains of MRSA on doubling the concentration of aqueous green tea extract from 100 mg/ml to 200 mg/ml there was no appreciable increase in antimicrobial activity and inhibition zone diameter remained almost same.

Amongst the 6 strains of MDRPA even at lowest concentration of 12.5 mg/ml the aqueous green tea extract showed appreciable antimicrobial activity against four strains (MDRPA-1, 2, 5, 6).

In the 4 strains (MDRPA 1,2,3,4) on increasing the concentration of the aqueous green tea extract from 12.5 mg/ml to 200 mg/ml there was a significant increase in inhibition zone diameter.

In case of MDRPA-5, even on increasing the concentration of aqueous green tea extract there was not much appreciable increase in antimicrobial activity and inhibition zone diameter remained almost same.

In case of MDRPA-6, even on doubling the concentration of aqueous green tea extract from 100 mg/ml to 200 mg/ml there was no increase in antimicrobial activity and inhibition zone diameter remained same.

From the findings of the study it is clearly evident that aqueous green tea extract has potent antimicrobial activity against MRSA, followed by Standard bacterial strains (ATCC) and then MDRPA strains.

Table 1: Antimicrobial activity of Aqueous Green Tea extract against Standard bacterial strains

Aqueous GT Extract	**Inhibition Zone Diameter (mm)**		
Concentration (mg/ml)	***E.coli* ATCC 25922**	***S.aureus* ATCC 25923**	***Pseudomonas aeruginosa* ATCC 27853**
200	20	26	22
100	17	24	20
50	14	15	15
25	12	14	15
12.5	8	10	10
Neg. Control (C)	0	0	0

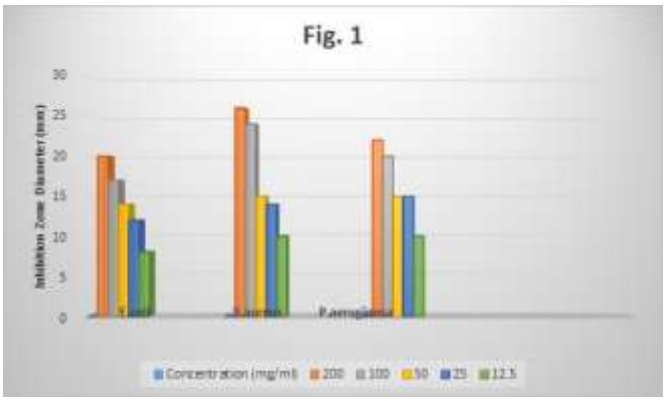


Fig. 1: Antimicrobial Activity of Aqueous Green Tea Extract against Standard Bacterial strains

Table 2: Antimicrobial activity of Aqueous Green Tea extract against Methicillin Resistant Staphylococcus aureus (MRSA)

Aqueous GT Extract	**Inhibition Zone Diameters (mm)**					
Concentration	**MRSA-1**	**MRSA-2**	**MRSA-3**	**MRSA-4**	**MRSA-5**	**MRSA-6**
200	23	19	30	21	22	12
100	22	18	30	18	22	11
50	18	17	27	14	18	10
25	15	12	19	8	17	9
12.5	13	10	19	0	10	0
Neg. Control (C)	0	0	0	0	0	0

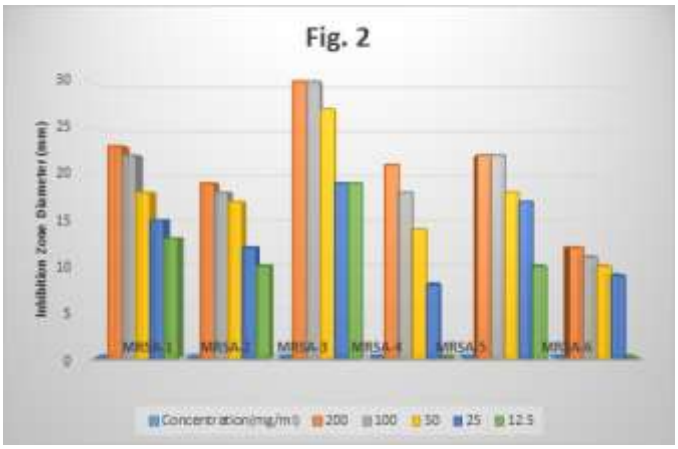


Fig. 2: Antimicrobial Activity of Aqueous Green Tea Extract against MRSA
MRSA- Methicillin Resistant Staphylococcus aureus

Table 3: Antimicrobial activity of Aqueous Green Tea extract against Multidrug Resistant Pseudomonas aeruginosa (MDRPA)

Aqueous GT Extract	Inhibition Zone Diameters(mm)					
Concentration	**MDRPA-1**	**MDRPA-2**	**MDRPA-3**	**MDRPA-4**	**MDRPA-5**	**MDRPA-6**
200	18	22	15	18	13	20
100	15	19	13	15	12	20
50	14	16	10	12	10	17
25	13	14	8	8	10	15
12.5	8	10	0	0	10	10
Neg. Control(C)	0	0	0	0	0	0

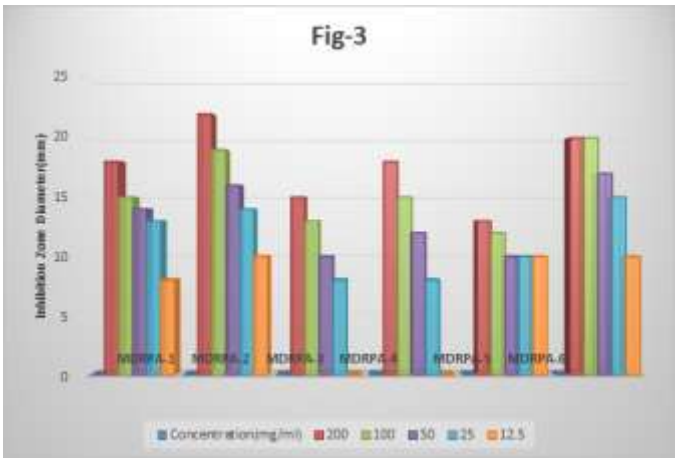


Fig. 3: Antimicrobial Activity of Aqueous Green Tea Extract against MDRPA
MDRPA-Multidrug Resistant Pseudomonas aeruginosa

Image 1: Antimicrobial Activity of Aqueous Green Tea Extract against Methicillin Resistant Staphylococcus aureus (MRSA)

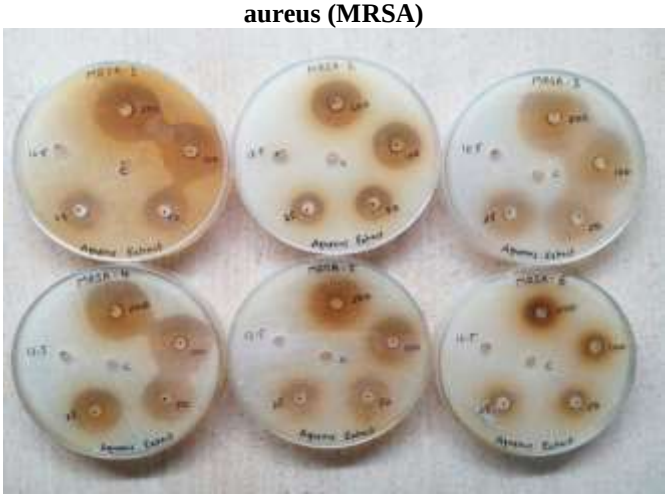
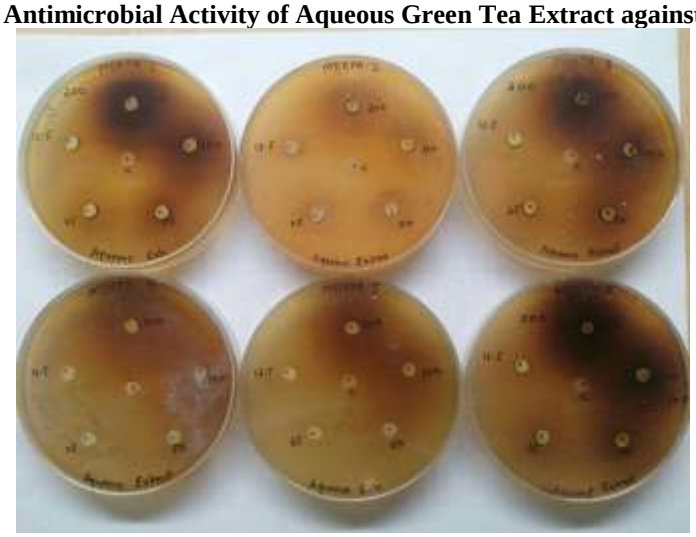


Image 2: Antimicrobial Activity of Aqueous Green Tea Extract against MDRPA



Discussion

The emergence of multi drug resistant strains of pathogenic bacteria is the biggest challenge in control of infectious diseases in communities as well as in hospital settings, in developing as well as in developed countries. A high percentage of Hospital acquired infections are caused by such highly resistant bacteria like MRSA and MDRPA.

With the emergence of multi drug resistance it is becoming increasingly difficult to treat these infections. Sooner or later these infections will become untreatable as no more alternatives will be left with clinicians[4]. To face this challenge there has been growing interest to explore new antimicrobial compounds from herbal extracts which could be incorporated as adjunctive therapy in such infections in near future[5].

Green tea (Camellia sinensis) has been reported to have modest antimicrobial activity against various pathogenic bacteria including MRSA and MDRPA[3,10].

It is clearly evident from the results of our study that the aqueous extract of commercial green tea (Camellia sinensis) prepared by cold extraction method has a potent antibacterial activity not only against standard ATCC bacterial strains but also against highly resistant pathogenic bacteria like MRSA.

The above observation is in accordance with the other studies conducted by Fanaki HN et. al[2], Radji M et.al[3],Cho YS et.al[10], Koech K.R. et.al[11], Jazani HN et.al[12] which have shown that green tea extract exhibit antimicrobial activity against highly resistant bacterial strains such as MRSA, MDRPA and other multi drug resistant Gram negative bacilli.

The antibacterial property of Green tea is mainly attributed to the polyphenolic components (esp. catechins) including Epicatechin, Epicatechin gallate, Epigallocatechin and Epigallocatechin gallate[3].

The direct antibacterial activity of tea catechins is the result of binding of catechins to the bacterial cell membrane causing damage to the membrane. This inhibits the ability of the bacterial to bind to host cells and also to bind to each other to from biofilms[3,13,14].

It has been found that the main polyphenolic component of green tea i.e. Epigallocatechin gallate can lead to reversal of methicillin resistance in MRSA by inhibiting synthesis of PBP2[3,13.15]

Green tea catechins have the ability to interfere with bacterial DNA replication by interacting with and thereby inhibiting the function of DNA gyrase[13].

Green tea polyphenols can inhibit the enzyme dihydrofolate reductase in bacteria and yeast effectively blocking the ability of microbes to synthesize purine and Pyramidine[13,16].

In this study we have assessed the antimicrobial activity of green tea extract but not studied its synergistic effect on antibiotics against multi drug resistant bacteria. Had it been included the utility of the study would have definitely increased. So, in future we will definitely conduct further studies to evaluate the synergistic or antagonistic effects of green tea extracts with antibiotics so as to further extend the scope of our research.

Conclusion

At the end of the study we have reached the conclusion that aqueous extract of Commercial Darjeeling green tea (*Camellia sinensis*) prepared by cold extraction method do have a significant antibacterial activity not only against standard ATCC bacterial strains but also against highly resistant clinical isolates of MRSA & MDRPA . This opens up a tremendous scope for the exploration of new antimicrobial compounds from herbal extracts such as the polyphenolic compounds (catechins) from the green tea which would be incorporated as adjuvant therapeutic agent in the treatment of multidrug resistant infections in community as well as hospital settings.

Conflict of Interest: None

Source of Support: Nil

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